

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 12-18 and 33 are pending in the application, with claims 12 and 33 being the independent claims. Claims 1-11, 19-32 and 34-43 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. Applicants respectfully assert that this amendment places the application in condition for allowance or in better form for consideration on appeal. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 12-18 and 33 were rejected under 35 U.S.C. § 112, first paragraph as allegedly not enabled. Specifically, the Examiner asserts that the specification does not provide support for a method of treating cancer with an antibody that binds to IGSF9. Applicants respectfully traverse the rejection.

In order to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, Applicants' specification must enable any person skilled in the art to make and use the claimed invention without undue experimentation. *See In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). *See also United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The factors to be considered when determining whether the necessary experimentation is

"undue" include: (a) the breadth of the claims, (b) the nature of the invention, (c) the state of the prior art, (d) the level of one of ordinary skill, (e) the level of predictability in the art, (f) the amount of direction provided by the inventor, (g) the existence of working examples, and (h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. Moreover, as long as the specification discloses at least one method for making and using the claimed invention, then the enablement requirement of 35 U.S.C. § 112, first paragraph is satisfied. *See Johns Hopkins Univ. v. CellPro, Inc.*, 152 F.3d 1342, 1361, 47 USPQ2d 1705, 1719 (Fed. Cir. 1998).

An Applicant is not limited to the confines of the specification to provide the necessary information to enable an invention. *See In re Howarth*, 654 F.2d 103, 105-6, 210 USPQ 689, 692 (CCPA 1981). Moreover, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed starting materials. *See Id.*

The Examiner again relies on several articles in support of the assertion of non-enablement, including Jain R.K. (*Scientific American*, 271:58-65 (1994)) (Jain), Dillman, R.O. (*Annals of Internal Medicine*, 111:592-603 (1989)) (Dillman I), Weiner, L.M. (*Seminars in Oncology*, 26(4 Suppl 12):41-50 (1999)) (Weiner) and Dillman, R.O. (*Journal of Clinical Oncology*, 12:1497-1515 (1994)) (Dillman II). As argued previously, Applicants respectfully assert that these articles are not indicative of the state of the art of the present invention because these articles were published a minimum of 4 years prior to the priority date of the present invention, and in one case nearly 9 years prior. In addition, as previously stated, the Weiner reference actually supports the use of

monoclonal antibody-based therapeutics in the treatment of cancer. Thus, the Examiner has not provided a single reference to support an argument that reflects the state of the art of the present invention. Thus, the Examiner has failed to establish a *prima facie* case of lack of enablement.

Even assuming, *arguendo*, that the Examiner has established a *prima facie* case, Applicants respectfully disagree with the Examiner's assertion that the problems associated with antibody therapy have not been overcome to date. Submitted herewith is a review article by Chung and Saltz that discusses antibody-based therapies for colorectal cancer (Exhibit A). The authors focus on two monoclonal antibodies and provide an in-depth analysis of the positive clinical data by both the antibodies for the treatment of patients with colorectal cancer. It is important to note that many of the references cited in Chung and Saltz were published near the time of filing the claimed invention. In addition, Kuroki *et al.* (Exhibit B) focuses on the increasingly successful application of monoclonal antibodies in the treatment of cancer. Therefore, Applicants respectfully assert that it would not require more than routine experimentation to use a monoclonal antibody of the present invention to treat one of the neoplastic disorders with which the specification discloses that IGSF9 is associated. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. § 102

Claim 9 was rejected under 35 U.S.C. § 102(e) as allegedly anticipated by Afar *et al.* (WO 2003/042661). Not in acquiescence to the propriety of the rejection, but rather solely to advance prosecution, Applicants have canceled claim 9. Therefore, the

rejection has been rendered moot and Applicants respectfully request that it be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 9-11 and 32 were rejected under 35 U.S.C. § 103 as allegedly unpatentable over Afar *et al.* (WO 2003/042661), in view of Chinn *et al.* (U.S. 6,994,840). Not in acquiescence to the propriety of the rejection, but rather solely to advance prosecution, Applicants have canceled claims 9-11 and 32. Therefore, the rejection has been rendered moot and Applicants respectfully request that it be withdrawn.


Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Paul A. Calvo
Attorney for Applicants
Registration No. 57,913

Date: January 16, 2007

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

631161_1.DOC

Antibody-Based Therapies for Colorectal Cancer

KI YOUNG CHUNG, LEONARD B. SALTZ

Gastrointestinal Oncology Service, Memorial Sloan-Kettering Cancer Center, New York, New York, USA;
Weill Medical College of Cornell University, New York, New York, USA

Key Words. Angiogenesis • Epidermal growth factor receptor • Monoclonal antibodies
Bevacizumab • Cetuximab

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Select the appropriate clinical use of cetuximab and bevacizumab in the treatment of metastatic colorectal cancer based on the currently available clinical trial data and known toxicities of each agent.
2. Discuss the controversy over EGFR testing in colorectal cancer patients and the lack of predictive value of EGFR expression by IHC.
3. List the relevant clinical questions regarding the use of targeted agents in colorectal cancer that remain to be addressed by clinical trials.



Access and take the CME test online and receive 1 hour of AMA PRA category 1 credit at CME.TheOncologist.com

ABSTRACT

The recent successful development of novel monoclonal antibodies that target key components of biologic pathways has expanded the armamentarium of treatment options for patients with colorectal cancer. Two targets in particular—the process of new blood vessel development, or angiogenesis, and the epidermal growth factor receptor and its signaling pathway—are exploited by the newest monoclonal antibodies that are

available for use in colorectal cancer patients. This clinical review focuses on the defining role of the two most clinically advanced novel agents, bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, <http://www.gene.com>) and cetuximab (Erbix®; ImClone Systems, Inc., New York, <http://www.imclone.com>), in colorectal cancer. *The Oncologist* 2005;10:701–709

INTRODUCTION

The recent successful development of novel monoclonal antibodies that target key components of biologic pathways has expanded the armamentarium of treatment options for patients with colorectal cancer. Conceptually, these newer agents are more scientifically appealing than older cytotoxic agents, as they more specifically target unique features of the cancer cell

and its surroundings and so attempt to exploit the progress that has been made in the understanding of basic cell biology. Two targets in particular—the process of new blood vessel development, or angiogenesis, and the epidermal growth factor receptor and its signaling pathway—are exploited by the newest monoclonal antibodies that are available for use in colorectal cancer patients. This clinical review focuses on the

Correspondence: Ki Young Chung, M.D., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA. Telephone: 212-639-6121; Fax: 646-422-2246; e-mail: chungk@mskcc.org Received April 11, 2005; accepted for publication July 25, 2005. ©AlphaMed Press 1083-7159/2005/\$12.00/0

The Oncologist 2005;10:701–709 www.TheOncologist.com

Downloaded from www.TheOncologist.com by on January 16, 2007

defining role of the two most clinically advanced novel agents, bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, <http://www.gene.com>) and cetuximab (Erbix®; ImClone Systems, Inc., New York, <http://www.imclone.com>), in colorectal cancer.

Monoclonal Antibodies

Monoclonal antibodies have traditionally been produced from hybridoma cells, which are immortalized mouse B cells derived from the fusion of B cells with myeloma cells. The major drawback to this method of antibody production is that the resultant antibody is a murine protein, and once injected into humans, murine antibodies are often highly antigenic, giving rise to a human anti-mouse antibody (HAMA) response [1]. The HAMA not only has the potential to result in an allergic response but also has the potential to neutralize the therapeutic antibody and so impair tumor targeting.

To circumvent these problems, researchers have developed a number of methods to make antibodies appear more human-like to the immune system. One approach has been to graft regions of human antibodies onto mouse antibodies, producing chimeric and humanized antibodies that can reduce the HAMA response, although this time-consuming process does not completely eliminate the immune reaction.

Another more recent technology is the use of the Xeno Mouse® (Amgen, Inc., Thousand Oaks, CA, <http://www.amgen.com>) to produce fully humanized antibodies [2]. This transgenic mouse has been genetically engineered by deleting murine immunoglobulin genes and replacing them with a repertoire of human antibody genes.

One antibody derived from the XenoMouse® is ABX-EGF, or panitumumab (Abgenix, Fremont, CA, <http://www.abgenix.com>). This fully humanized antibody targets the epidermal growth factor receptor (EGFR), which mediates growth signals that stimulate cell growth.

In addition to blocking or stimulating their targets, some monoclonal antibodies may work by inducing complement-dependent cytotoxicity and antibody-dependent cell cytotoxicity (ADCC). These multiple potential mechanisms of action may be at least in part responsible for the differences in clinical trial results of monoclonal antibodies in comparison with small molecule inhibitors (e.g., erlotinib [Tarceva®; OSI Pharmaceuticals, Inc., Melville, NY, <http://www.osip.com>], gefitinib [Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE, <http://www.astrazeneca-us.com>]) that target the same pathway.

Bevacizumab

Bevacizumab is a humanized monoclonal antibody that targets and binds to vascular endothelial growth factor-A

(VEGF-A), reducing the availability of VEGF ligand for its intended receptor (R1-2) and thereby preventing receptor activation [3]

Kabbinnavar et al. reported the first clinical trial of bevacizumab in combination with 5-fluorouracil and leucovorin (5-FU/LV) in previously untreated colorectal cancer patients [4]. This small phase II trial involved 104 patients randomized to two different dose levels of bevacizumab (5 mg/kg or 10 mg/kg) plus weekly 5-FU and high-dose LV (Roswell Park 5-FU/LV schedule) or to 5-FU/LV alone [4]. Despite the small study size, the response rate (RR), time to tumor progression (TTP), and overall survival (OS) were superior in the 5-FU/LV + 5 mg/kg bevacizumab arm, with the 10-mg/kg arm appearing modestly superior to chemotherapy alone but inferior to the low-dose 5-mg/kg arm (Table 1). Thrombosis was the most significant adverse event, with five patients in the 5-mg/kg arm and two patients in the 10-mg/kg arm developing a grade 3–4 thrombosis, with one fatality in the 10-mg/kg arm resulting from a pulmonary embolism. Hypertension, proteinuria, and epistaxis were also seen but were relatively infrequent. This randomized phase II trial provided enough preliminary evidence of activity of bevacizumab in combination with 5-FU/LV to justify a definitive phase III trial and suggested that 5 mg/kg every other week would be the appropriate dose to take forward for further development.

The initial plan had been to compare 5-FU/LV plus placebo with 5-FU/LV plus 5 mg/kg of bevacizumab in a phase III trial. However, as the above-mentioned phase II trial was being completed, a randomized phase III trial was reported by Saltz et al. that demonstrated a modest but statistically significant survival advantage for the IFL regimen (irinotecan [Camptosar®; Pfizer Pharmaceuticals, New York, <http://www.pfizer.com>] plus weekly bolus 5-FU/LV) over 5-FU/LV alone [5]. At the time, however, there were no safety data on the combination of bevacizumab plus IFL. For this reason, a three-arm clinical trial was designed: 5-FU/LV/bevacizumab, IFL/bevacizumab, and IFL/placebo (the control arm) [6]. A planned safety analysis was incorporated into the trial design when enrollment reached 100 patients in each arm. As per the study plan, the 5-FU/LV/bevacizumab arm was closed at that point because safety analyses indicated acceptable tolerability of the IFL/bevacizumab arm.

No crossover to second-line bevacizumab in the IFL/placebo control arm was permitted. In the final efficacy analysis reported by Hurwitz et al. [6], the IFL/bevacizumab cohort experienced superior outcome to IFL/placebo in every major trial end point: RR (45% versus 35%; $p < .003$), progression-free survival (PFS) (10.6 months versus 6.2 months; $p < .00001$), and OS (20.3 months versus 15.6 months, $p = .00003$) (Table 2).

The most common reported toxicity in the IFL/bevacizumab arm was moderate hypertension, which was treatable with minimal pharmacologic intervention (22% in the IFL/bevacizumab arm versus 8.3% in the IFL alone arm). Specifically, grade 3 hypertension was higher in the bevacizumab/IFL arm than in the placebo/IFL arm (11% versus 2%). Incidences of thromboembolic events overall and proteinuria were indistinguishable between arms.

Two rare but extremely serious toxicities were encountered, however. The first was gastrointestinal (GI) perforation. This was a heterogeneous group of events that included a perforated gastric ulcer, small bowel perforations, and free air under the diaphragm without an identified source. Six events characterized as GI perforation occurred in patients on the bevacizumab-containing arm (one fatal) compared with none on the chemotherapy-alone arm. No clear risk factors for perforation could be identified from this trial. The other issue of serious concern was that of arterial thrombotic events. Initially, no clear safety signal was detected in this area. However, when a subsequent analysis involving patients from multiple trials was conducted, a concerning pattern was detected. The total combined incidence of such events, which were defined as

including cerebral vascular accidents, myocardial infarctions, transient ischemic attacks, and angina, was 2.5% in the nonbevacizumab-containing control arms and 5.0% in the bevacizumab-containing experimental arms. It was initially noted that patients with histories of cardiovascular and/or atherosclerotic disease appeared to be at greater risk for more bevacizumab-related arterial thrombotic complications.

In addition to the pivotal phase III trial, another phase II trial also demonstrated activity of bevacizumab in the first-line treatment of metastatic colorectal cancer [7]. This smaller trial, designed for those patients in whom irinotecan-based therapy was deemed inappropriate by the treating investigator, randomized patients to 5-FU/LV (Roswell Park Schedule) with placebo or 5 mg/kg of bevacizumab every other week (Table 1). While the study lacked adequate power to assess a survival advantage, superior response rates and TTP were seen with the bevacizumab-containing arm.

Not convinced of the appropriateness of the 5-mg/kg bevacizumab dose, the Eastern Cooperative Oncology Group (ECOG) conducted a phase II trial (E2200) of IFL plus bevacizumab at 10 mg/kg [8, 9]. Ninety-two previously

Table 1. Bolus 5-FU and bevacizumab randomized trials

Treatment arm	No. of patients	Response rate	Median time to progression	Median survival
Kabbinnavar et al. [4]				
5-FU/LV	35	17%	5.2 months	13.6 months
5-FU/LV + bevacizumab (5 mg/kg)	35	40% ^a	9.0 months	7.2 months
5-FU/LV + bevacizumab (10 mg/kg)	32	24%	7.2 months	16.1 months
Kabbinnavar et al. [7]				
5-FU/LV + placebo	105	15%	5.5 months	12.9 months
5-FU/LV + bevacizumab (5 mg/kg)	104	26% ^b	9.2 months ^c	16.6 months ^d

^a*p* = .029; ^b*p* = .0552; ^c*p* = .0002; ^d*p* = .159.

Abbreviations: 5-FU, 5-fluorouracil; LV, leucovorin.

Table 2. IFL + bevacizumab trials

Treatment arm (study)	No. of patients	Overall response rate	Progression-free survival (months)	1-year survival (%)	Median overall survival (months)
IFL + bevacizumab (E2200 trial)	92	49%	10 (8.4–12.2)	85%	Not reached
IFL + placebo (Hurwitz et al. [6] phase III trial)	411	35%	6.2	63.4%	15.6
IFL + bevacizumab (Hurwitz et al. [6] phase III trial)	402	45% ^a	10.6 ^b	74.3%	20.3 ^b

^a*p* = .004; ^b*p* < .001.

Abbreviation: IFL, irinotecan, 5-fluorouracil, and leucovorin.

untreated metastatic colorectal cancer patients were treated. With a median follow-up of 16.7 months, five complete responses (CRs) (5%) and 35 partial responses (PRs) (38%) were reported, with OS yet to be reached (Table 2). Preliminary safety data from 87 patients revealed 47 bleeding events, but only four events of grade ≥ 2 . Eleven thrombotic episodes were reported, none fatal. It is difficult to know what we should glean from this trial. The 42% response rate does not appear to be different from (or superior to) the 45% response rate seen with the 5-mg/kg dose in the pivotal trial. Overall, the use of a dose < 5 mg/kg dose does not appear to be justified in first-line treatment at this time.

Recently, the use of bevacizumab in second-line therapy in those patients who have *not* received bevacizumab in the first-line setting has been established by the phase III ECOG trial E3200 [10]. That trial randomized patients who had failed irinotecan and fluorouracil but were naïve to bevacizumab, to one of three arms: bevacizumab/FOLFOX4 (5-FU/LV/oxaliplatin [Eloxatin®; Sanofi-Synthelabo Inc., New York, <http://www.sanofi-synthelabo.us>]), FOLFOX4 alone, or bevacizumab. Again, ECOG chose to investigate a 10-mg/kg bevacizumab dose. A total of 829 patients was accrued from November 2001 to April 2003, with the bevacizumab monotherapy arm closed in March 2003 by the data safety monitoring committee, reportedly because of inferior activity. The overall RR and median PFS in the bevacizumab/FOLFOX4 arm were clearly superior to those of the FOLFOX4 and bevacizumab-alone arms (RR, 21.8% versus 9.2% versus 3%, respectively; $p < .0001$; PFS, 7.2 months versus 4.8 months versus 2.7 months, respectively; $p < .0001$) [11]. A small but statistically significant longer median overall survival was demonstrated in the combination arm compared with FOLFOX4 alone (12.5 months versus 10.7 months; $p = .0024$), and the incidences of grade 3–4 toxicities were not greater. Interestingly, the median OS times in the FOLFOX4 arm and the bevacizumab-alone arm were indistinguishable (10.7 months versus 10.2 months, respectively; $p = .95$) [11].

The safety of bevacizumab in combination with oxaliplatin was further addressed by the recently reported Three Regimens of Eloxatin® Evaluation-2 (TREE-2) study, a randomized phase II study of three oxaliplatin–fluoropyrimidine regimens in the first-line therapy of colorectal cancer [12]. The authors of that trial reported that the addition of bevacizumab to an oxaliplatin-containing regimen of infusional 5-FU (FOLFOX) or bolus 5-FU/LV (bFOL) or to capecitabine (Xeloda®; Hoffmann-La Roche Inc., Nutley, NJ, <http://www.rocheusa.com>) (cape/ox) appeared to produce a superior overall response rate (RR, 57% for FOLFOX; 95% confidence interval [CI], 37%–75%) relative to historic controls from an earlier trial (TREE-1) of

FOLFOX alone, with no significant additive toxicity. In an attempt to address the benefit of bevacizumab to more current first-line combinations, a Southwest Oncology Group (SWOG) trial was initiated to compare FOLFOX with CapOx (capecitabine and oxaliplatin) with or without bevacizumab in a 2×2 factorial design; however, the trial was forced to close secondary to poor accrual once bevacizumab became commercially available in the U.S. A large industry-sponsored trial has randomized 1,600 patients to front-line FOLFOX4 versus CapOx, with a 2×2 randomization to bevacizumab versus placebo. That trial has completed accrual and data are maturing.

CURRENT PRACTICE RECOMMENDATIONS

Based on the conclusions of multiple clinical trials [5, 13, 14], neither the weekly bolus IFL nor the Roswell Park 5-FU/LV regimen would be the front-line chemotherapy regimen of choice for most patients at this time. Hence, it would seem to be an appropriate practice to use an appropriate first-line cytotoxic combination regimen such as either 5-FU/LV/irinotecan (FOLFIRI) or FOLFOX along with the addition of bevacizumab (5 mg/kg) every other week to either regimen. The data for efficacy of first-line bevacizumab are compelling, and short of a specific significant contraindication, bevacizumab should be considered for incorporation into the front-line management of patients with colorectal cancer.

An important question that remains unresolved at this time is whether to continue bevacizumab with second-line therapy following failure of a bevacizumab-containing first-line regimen. The ECOG 3200 study supports the position that patients who have not received bevacizumab with front-line therapy should receive it as part of their second-line therapy. However, that trial provided no data regarding the question of using bevacizumab in second-line therapy if the patient has failed front-line therapy that includes bevacizumab. A theoretical justification for the continuation of bevacizumab after failure can be made on preclinical grounds, invoking the hypothesis that bevacizumab may normalize tumor interstitial fluid pressure and thereby enhance chemotherapy delivery, as well as possibly inhibit the neovascularization and consequent vascular density of tumors. If the major role of bevacizumab is to normalize tumor interstitial fluid pressure and thereby improve chemotherapy delivery, then failure of clinical response with a bevacizumab-containing regimen could be a reflection of nonresponsiveness to the cytotoxic chemotherapeutic agent and not to bevacizumab. Hence, one could anticipate that bevacizumab might be effective when given with another effective cytotoxic agent. This is a hypothesis worthy of exploration. However, it is just that: a hypothesis. Standard practice should be dic-

tated on evidence-based decisions, not on unsubstantiated hypotheses. A clinical trial addressing the potential benefit of continued bevacizumab with a new chemotherapy regimen after bevacizumab failure is warranted. Until such data are available, however, the risk and expense of continued bevacizumab do not seem justifiable, and continuation of bevacizumab after failure on a bevacizumab regimen should be regarded as investigational. Prospective randomized clinical trials to address this issue have been proposed, but thus far these trials have not been initiated.

The use of bevacizumab as a salvage agent after all other active chemotherapy has failed, even in bevacizumab-naïve patients, does not appear to be supported by current data, and the bevacizumab-naïve patient in the salvage setting should become a much less common phenomenon, as most patients will have received bevacizumab in the first- or second-line setting based on available data from clinical phase III trials [6, 10]. In a trial conducted by the U.S. National Cancer Institute, 350 patients with prior failure on 5-FU, irinotecan, and oxaliplatin (in any combination of regimens) were treated with 5-FU, LV, and bevacizumab [15]. A preliminary report at the American Society of Clinical Oncology meeting in June 2004 on the first 100 patients treated indicated that one of the 100 patients had achieved a major objective response. This would suggest that activity as a salvage agent in the absence of active chemotherapy is negligible, and the routine use of bevacizumab in this salvage setting is not recommended. The 1% RR must be weighed against the 1.5% GI perforation rate and the 2.5% higher incidence of arterial thrombotic events.

Currently, there is no evidence to support the use of bevacizumab in the adjuvant setting, although trials to assess this question are in progress. A U.S. follow-up of the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colorectal Cancer (MOSAIC) trial, a large phase III randomized adjuvant clinical trial by the National Surgical Adjuvant Breast and Bowel Project for stage II/III colorectal patients is comparing FOLFOX6 with FOLFOX6 plus bevacizumab, with the bevacizumab continued for an additional 6 months alone. A large European trial will randomize patients to one of three arms: FOLFOX4/placebo, FOLFOX4/bevacizumab, and CapOx/bevacizumab. Pending data from these trials, the use of bevacizumab in stage II and III colon cancer should be regarded as investigational and cannot be recommended for routine standard use at this time.

Cetuximab

Cetuximab is a chimeric immunoglobulin G₁ monoclonal antibody that targets the extracellular domain of the EGFR, competitively inhibiting ligand binding and, hence, EGFR

activation [16]. Several recent trials have demonstrated the clinical activity of cetuximab in metastatic colorectal cancer. The EGFR, also called HER-1, is a transmembrane glycoprotein that binds specific ligands, EGF and transforming growth factor alpha, to the extracellular domain, leading to dimerization of the receptor with another EGFR (homodimerization) or another member of the EGFR family (heterodimerization). This in turn stimulates phosphorylation of the intracellular tyrosine kinases of the receptor, initiating a cascade of intracellular signaling that ultimately regulates cell proliferation, migration, adhesion, differentiation, and survival [17–19]. Preclinical models demonstrated modest *in vitro* and *in vivo* single-agent activity of cetuximab but significant enhancing activity in combination with cytotoxic chemotherapy. Based on these observations, and on a single anecdotal report of a major response to cetuximab plus irinotecan in a young irinotecan-refractory patient, a multicenter phase II trial was initiated and has been reported in abstract form [20]. Patients were treated with the same dose and schedule of irinotecan that they had previously failed, with the addition of cetuximab at the current standard of a 400-mg/m² loading dose week 1 over 2 hours, followed by weekly doses of 250 mg/m² over 1 hour. Irinotecan dose reductions made prior to study entry were maintained upon initiation of the trial. One hundred twenty patients with irinotecan-refractory colorectal cancer were identified and enrolled. An additional 28 patients with clinically and radiographically stable disease after receiving a minimum of 3 months of irinotecan therapy were also enrolled and treated by the addition of cetuximab to their irinotecan therapy. The response outcome of this “stable disease cohort” has not been reported. A 22.5% major objective RR for irinotecan-refractory patients was reported by an independent response assessment committee (Table 3). The reported irinotecan toxicity was, not unexpectedly, modest in this population in whom irinotecan dose modifications had already been made during prestudy treatment. Only 3% of patients developed an allergic, anaphylactoid reaction requiring discontinuation of cetuximab therapy. Seventy-five percent of patients treated experienced a skin rash (12% grade 3), characteristic of all EGFR inhibitors. This rash superficially resembles acne, leading to its description as an acneiform rash; however, it is pathophysiologically distinct and, hence, topical acne medications are not effective in its management. The presence of the rash appeared to be associated with response in this study, but this needs to be validated in larger trials.

The encouraging results seen in the phase II cetuximab plus irinotecan trial led to the initiation of a multicenter phase II trial to evaluate the activity of single-agent cetuximab in irinotecan-refractory colorectal cancer [21]. In that

trial, 5 of 57 patients (9%) achieved PRs confirmed by an independent radiologic review (Table 3).

A subsequent, larger trial reported by Cunningham et al. [22] provided strong confirmatory evidence of the activity of cetuximab in colorectal cancer. This was a large, randomized phase II trial in irinotecan-refractory patients comparing cetuximab plus irinotecan with cetuximab monotherapy. Three hundred twenty-nine patients were randomized in a 2:1 schema. The RRs of 22.9% for cetuximab plus irinotecan and 10.8% for cetuximab alone were virtually identical to the RRs reported in the U.S. phase II trials. TTP in the Cunningham et al. study was 4.1 months for the combination versus 1.5 months for single-agent cetuximab. Survival in the two arms was not significantly different (Table 3).

Addressing the role of second-line cetuximab in irinotecan-naïve disease, a randomized phase III trial (CA225-006) is comparing cetuximab plus irinotecan with irinotecan alone as second-line chemotherapy for patients with EGFR-positive metastatic colorectal cancer progressing on an oxaliplatin/5-FU-based regimen. A palliative National Cancer Institute of Canada Clinical Trial Group phase III clinical trial is comparing cetuximab plus best supportive care with best supportive care alone in patients for whom best supportive care is the only remaining treatment option. Again, EGFR positivity is mandatory for eligibility in this clinical trial. A total of 500 patients is expected for accrual, with treatment to continue in the absence of disease progression or unacceptable toxicity.

Cetuximab has established activity in the salvage setting; however, its role in first-line therapy remains investigational at this time. Preliminary data from phase II trials have reported encouraging results, however. A small phase

II pilot trial of cetuximab plus weekly bolus IFL demonstrated a 44% RR [23]. A phase II experience of cetuximab plus irinotecan and weekly infusional 5-FU/LV has also been reported [24]. A phase II trial of FOLFOX4 plus cetuximab for previously untreated metastatic colorectal cancer patients reported preliminary encouraging activity [25]. Of 43 patients enrolled, 42 evaluable, 4 CRs and 30 PRs were reported for a RR of 81%, without a higher incidence of grade 3–4 diarrhea or neutropenia compared with historical controls. A phase I/II trial of cetuximab plus 5-FU/LV (two dose cohorts) and weekly oxaliplatin (50 mg/m²) (FUFOX) in first-line treatment has reported preliminary data from 38 of 49 enrolled patients [26]. One CR and 20 PRs were reported with an overall RR of 55%. The 41 patients evaluable at dose level 2 reported significant grade 3–4 diarrhea (24%) and skin reactions (17%), and preliminarily 7% grade 3–4 neurotoxicity. Additionally, a phase I/II trial of FOLFIRI plus cetuximab in first-line therapy with two dose levels of 5-FU resulted in encouraging preliminary data [27]. Of 40 evaluable patients, a 43% PR rate (95% CI, 27%–59%) was reported, with five initially unresectable patients undergoing an R0 resection and with minimal grade 3–4 toxicities (17% leukopenia, 14% diarrhea, 11% nausea and vomiting, 7% asthenia, 7% skin reaction).

Thus far, no randomized trials of first-line cetuximab plus chemotherapy have been reported. As such, no data are available on what impact, if any, this drug will have on survival and other efficacy end points in first-line combinations. Several such trials are in progress or in the planning stages, but given the cutaneous toxicity as well as the significant economic cost of extended cetuximab treatment, front-line use of cetuximab must be regarded as investigational at this time. The role of cetuximab in adjuvant ther-

Table 3. Cetuximab trials

Treatment arm (study)	Patients	Overall response rate (%)	Disease control (%)	Median time to progression (months)	Median overall survival (months)
Cetuximab (Saltz et al. [21])	57	11 (4–22)	33	1.4	6.4
Cetuximab (Cunningham et al. [22])	111	11 (6–18)	32 (24–42)	1.5	6.9 (5.6–9.1)
Cetuximab + irinotecan (Saltz et al. [20])	121	17 (11–25)	48	NA	NA
Cetuximab + irinotecan (Cunningham et al. [22])	218	23 (18–29) ^a	56 (49–62) ^b	4.1 ^c	8.6 (7.6–9.6)

^a $p = .0074$; ^b $p = .0001$; ^c $p < .0001$.
Abbreviation: NA, not available.

apy of stage III colon cancer is also being investigated, but again this remains a study question and not part of standard care at this time.

A multicenter phase III clinical trial is currently comparing FOLFOX4 plus cetuximab with FOLFOX4 alone in irinotecan-refractory colorectal cancer patients. Similar to all the phase II trials to date, EGFR positivity by immunohistochemistry (IHC) is a mandatory eligibility requirement.

The development of cetuximab in colorectal cancer was grounded on the premise that EGFR expression by IHC would be prognostic for cetuximab activity, with all trials to date requiring EGFR positivity by IHC. Yet all the reported cetuximab trials demonstrate no correlation between intensity of EGFR expression and clinical response, challenging this premise [21, 22]. Lenz et al. reported, in abstract form, the results of a small cohort of nine EGFR-negative patients who were entered into a clinical trial of single-agent cetuximab [28]. Two major objective responses were reported by the investigators. On this basis, a decision was made at Memorial Sloan-Kettering Cancer Center that EGFR-negative colorectal cancer patients would not be excluded from standard off-protocol treatment with cetuximab simply on the basis of EGFR status. The retrospective institutional review of these patients treated in the first 3 months of cetuximab commercial availability identified 16 chemotherapy-refractory, EGFR-negative colorectal cancer patients who received cetuximab in a nonstudy setting [29]. Fourteen of those patients received cetuximab plus irinotecan and two received cetuximab monotherapy. Of the 16, four major objective responses were seen (RR, 25%; 95% CI, 4%–46%). This institutional series demonstrates that colorectal cancer patients with EGFR-negative tumors have the potential to respond to cetuximab-based therapies. EGFR analysis by current IHC techniques does not appear to have predictive value, and selection or exclusion of patients for cetuximab therapy on the basis of currently available EGFR IHC does not appear warranted.

Bevacizumab Plus Cetuximab

The Bowel Oncology with Cetuximab Antibody (BOND)-2 trial addressed the feasibility and, in a preliminary manner, efficacy of bevacizumab added to cetuximab alone or to cetuximab plus irinotecan in patients with irinotecan-refractory disease [30]. Preliminary data were encouraging, with 23% and 38% RRs, and 6.9-month and 8.5-month TTPs, respectively, which appear significantly better than those of historical controls from the BOND-1 trial (RR, 11% and 23%; TTP, 1.5 months and 4 months) [22, 30]. It should be noted, however that this is a small trial, with 39 and 35 patients, respectively, reported in the two arms in

this preliminary report. Ninety percent of the patients in this study also previously received oxaliplatin, making the combination an effective salvage regimen. No unexpected grade 3–4 toxicities were discovered.

An intergroup trial (Cancer and Leukemia Group B [CALGB]/SWOG 80405) will attempt to address the role of cetuximab and bevacizumab in first-line therapy for colorectal cancer. Investigators will choose which initial chemotherapy regimen (modified FOLFOX6 or FOLFIRI) will be used, and patients will then be randomized to receive either cetuximab, bevacizumab, or both agents together in conjunction with their chemotherapy. A follow-up study to the BOND-2 study, known as the BOND-2.5 trial, will assess the activity of the cetuximab–bevacizumab–irinotecan combination in patients who have previously progressed through a bevacizumab-containing regimen.

Other EGFR-Targeting Agents

Panitumumab, formally known as ABX-EGF, is a fully humanized monoclonal antibody that also targets the EGFR. Similar to cetuximab trials, preliminary data from a phase II trial of panitumumab in colorectal cancer patients reported a 10% RR, with over 90% of patients experiencing some degree of acneiform rash, although only 3% with grade ≥ 3 [31]. Only one of the 148 patients treated experienced a dose-limiting allergic reaction, supporting the contention that a humanized monoclonal antibody would elicit a lower incidence of allergic reactions than the chimeric monoclonal antibody cetuximab. Further clinical studies, including chemotherapy plus panitumumab trials, are yet to be reported.

EMD 72000, another fully humanized monoclonal antibody against the EGFR, has also demonstrated some antitumor activity in colorectal cancer patients in early clinical trials. The reported phase I trial enrolled 22 patients, 11 patients with heavily pretreated colorectal adenocarcinoma [32]. Two of the 11 enrolled colorectal cancer patients experienced a confirmed PR, with no grade 3 toxicities reported. Although encouraging, responses should be further validated in phase II trials. Thus far, the limited experience with the oral EGFR tyrosine kinase inhibitors gefitinib and erlotinib has not been encouraging in colorectal cancer, with no significant single-agent activity shown for either of these agents [33, 34]. A single-center, open-label phase II trial of FOLFOX4 plus gefitinib reported a 77% objective response rate in 30 evaluable patients [35]. However, the wide 95% CIs of such a small study and the significant toxicity reported, including 54% grade 3–4 diarrhea and 52% grade 3–4 neutropenia, makes the combination prohibitive at the dose levels used in the trial.

Conclusion

The integration of biologically targeted agents with conventional cytotoxic chemotherapy has already significantly impacted the survival of patients with advanced colorectal cancer. Through improvements in selecting responsive patients based on the molecular biology and behavior of colorectal cancers, we may eventually individualize the optimal therapeutic approach for each patient.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Dr. Saltz has acted as a consultant for Genentech, Sanofi, Pfizer, and Taiho within the past 2 years; has performed contract work for ImClone, Bristol-Myers Squibb, Pfizer, Roche, and Taiho within the past 2 years; and has received support from Genentech, Sanofi, Pfizer, and Taiho within the past 2 years. Dr. Chung has performed contract work for Taiho within the past 2 years.

REFERENCES

- Klee GG. Human anti-mouse antibodies. *Arch Pathol Lab Med* 2000;124:921-923.
- Green LL. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. *J Immunol Methods* 1999;231:11-23.
- Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 2004;25:581-611.
- Kabbinavar F, Hurwitz H, Fehrenbacher L et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003;21:60-65.
- Saltz LB, Cox JV, Blanke C et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000;343:905-914.
- Hurwitz H, Fehrenbacher L, Novotny W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335-2342.
- Kabbinavar F, Schultz J, McCleod M et al. Bevacizumab (a monoclonal antibody to vascular endothelial growth factor) to prolong progression-free survival in first line colorectal cancer (CRC) in subjects who are not suitable candidates for first-line CPT-11. Presented at the American Society of Clinical Oncology Gastrointestinal Symposium, San Francisco, California, January 22-24, 2004.
- Giantonio B, LD, Catalano P. Incorporating angiogenesis inhibition with bevacizumab (anti-VEGF) into front-line chemotherapy with irinotecan (CPT-11), fluorouracil and leucovorin (FU/LV) for advanced colorectal cancer (advCRC): a toxicity analysis of ECOG E2200. Presented at the American Society of Clinical Oncology Annual Meeting, Orlando, Florida, May 18-23, 2002.
- Giantonio B, Levy D, O'Dwyer PJ et al. Bevacizumab (anti-VEGF) plus IFL (irinotecan, fluorouracil, leucovorin) as front-line therapy for advanced colorectal cancer (advCRC): updated results from the Eastern Cooperative Oncology Group (ECOG) Study E2200. Presented at the American Society of Clinical Oncology Gastrointestinal Symposium, San Francisco, CA, January 22-24, 2004.
- Giantonio BJ, Catalano PJ, Meropol NJ et al. High-dose bevacizumab in combination with FOLFOX-4 improves survival in patients with previously treated advanced colorectal cancer: results from the Eastern Cooperative Oncology Group (ECOG) study E3200. Presented at the American Society of Clinical Oncology Gastrointestinal Symposium, Hollywood, Florida, January 27-29, 2005.
- Giantonio B, Catalano PJ, Meropol NJ et al. High-dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: results from the Eastern Cooperative Oncology Group (ECOG) study E3200. Presented at the 2005 American Society of Clinical Oncology Annual Meeting, Orlando, Florida, May 13-17, 2005.
- Hochster H, Welles L, Hart L et al. Bevacizumab (B) with oxaliplatin (O)-based chemotherapy in the first-line therapy of metastatic colorectal cancer (mCRC): preliminary results of the randomized "TREE-2" trial. Presented at the American Society of Clinical Oncology Gastrointestinal Symposium, Hollywood, Florida, January 27-29, 2005.
- Goldberg RM, Sargent DJ, Morton RF et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2004;22:23-30.
- Tournigand C, Andre T, Achille E et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;22:229-237.
- Chen H, Mooney M, Boron M et al. Bevacizumab (BV) plus 5-FU/leucovorin (FU/LV) for advanced colorectal cancer (CRC) that progressed after standard chemotherapies: an NCI Treatment Referral Center trial (TRC-0301). Presented at the 2004 American Society of Clinical Oncology Annual Meeting, New Orleans, Louisiana, June 5-8, 2004.
- Thomas SM, Grandis JR. Pharmacokinetic and pharmacodynamic properties of EGFR inhibitors under clinical investigation. *Cancer Treat Rev* 2004;30:255-268.
- Carpenter G, Cohen S. Epidermal growth factor. *J Biol Chem* 1990;265:7709-7712.
- Real FX, Rettig WJ, Chesa PG et al. Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation. *Cancer Res* 1986;46:4726-4731.
- Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001;7:2958-2970.
- Saltz LB, Rubin M, Hochster H et al. Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer (CRC) that express epidermal growth factor receptor (EGFR). *Proc Am Soc Clin Oncol* 2001;20:3a.
- Saltz LB, Meropol NJ, Loehrer PJ Sr et al. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;22:1201-1208.
- Cunningham D, Humblet Y, Siena S et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337-345.
- Rosenberg A, Loehrer PJ, Needle M et al. Erbitux (IMC-C225) plus weekly irinotecan (CPT-11), fluorouracil (5FU) and leucovorin (LV) in colorectal cancer (CRC) that expresses the epidermal growth factor receptor (EGFR). Presented at the 2002 American Society of Clinical Oncology Annual Meeting, May 18-23, 2002.

- 24 Van Laethem JL, Raoul JL, Mitry E et al. Cetuximab (C225) in combination with bi-weekly irinotecan (CPT-11), infusional 5-fluorouracil (5-FU) and folinic acid (FA) in patients (pts) with metastatic colorectal cancer (CRC) expressing the epidermal growth factor receptor (EGFR). Preliminary safety and efficacy results. *Proc Am Soc Clin Oncol* 2003;22:264.
- 25 Badarinath SME, Jennis A. Cetuximab plus FOLFOX for colorectal (EXPLORE): preliminary safety analysis of a randomized phase III trial. Presented at the 2004 American Society of Clinical Oncology Annual Meeting, New Orleans, Louisiana, June 5–8, 2004.
- 26 Hohler T. Phase I/II study of cetuximab combined with 5-fluorouracil/leucovorin plus weekly oxaliplatin in first-line treatment of epidermal growth factor receptor-expressing metastatic colorectal cancer. Presented at the European Society of Medical Oncology Congress, Vienna, Austria, October 29–November 2, 2004.
- 27 Rougier P, Raoul JL, Van Laethem JL et al. Cetuximab + FOLFIRI as first-line treatment for metastatic colorectal cancer. Presented at the 2004 American Society of Clinical Oncology Annual Meeting, New Orleans, Louisiana, June 5–8, 2004.
- 28 Lenz H, Mayer RJ, Gold PJ et al. Activity of cetuximab in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. *Proc Am Soc Clin Oncol* 2004;22:2475.
- 29 Chung KY, Shia J, Kemeny NE et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2005;23:1803–1810.
- 30 Saltz L, Lenz H, Kindler H et al. Interim report of randomized phase II trial of cetuximab/bevacizumab/irinotecan (CBI) versus cetuximab/irinotecan (CB) in irinotecan-refractory colorectal cancer. Presented at the American Society of Clinical Oncology Gastrointestinal Symposium, Hollywood, Florida, January 27–29, 2005.
- 31 Hecht J, Patnaik A, Malik I et al. ABX-EGF monotherapy in patients (pts) with metastatic colorectal cancer (mCRC): an updated analysis. Presented at the 2004 American Society of Clinical Oncology Annual Meeting, New Orleans, Louisiana, June 5–8, 2004.
- 32 Vanhoefer U, Tewes M, Rojo F et al. Phase I study of the humanized anti-epidermal growth factor receptor monoclonal antibody EMD72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. *J Clin Oncol* 2004;22:175–184.
- 33 Dorligschaw OKT, Jordan K. ZD1839 (Iressa)-based treatment as last-line therapy in patients with advanced colorectal cancer (ACRC). *Proc Am Soc Clin Oncol* 2003;22:372.
- 34 Oza ATC, Siu L. Phase II study of erlotinib (OSI-774) in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 2003;22:196.
- 35 Cho CFG, Halsey J. A phase II study of gefitinib in combination with FOLFOX-4 (IFOX) in patients with unresectable or metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 2003;22:265.

Possible Applications of Antibodies or their Genes in Cancer Therapy

MASAHIDE KUROKI^{1,3}, JIAN HUANG¹, HIROTOMO SHIBAGUCHI^{1,3}, TOSHIHIRO TANAKA¹, JUN ZHAO³, NAXIANG LUO¹, KEN HACHIMINE¹, TETSUSHI KINUGASA¹, SHIN-ICHI MAEKAWA¹, SOTARO ENATSU¹, WAKAKO HAMANAKA¹, TATSUYA FUKAMI² and MOTOMU KUROKI^{1,3}

¹Department of Biochemistry and ²Department of Obstetrics and Gynecology,
School of Medicine, Fukuoka University, Jonan-ku, Fukuoka;

³Fukuoka University Molecular Oncology Center, Jonan-ku, Fukuoka, 814-0180, Japan

Abstract. In this review article the possible applications of anti-tumor-associated antigen (TAA) antibodies in the therapy of cancer have been summarized. First, recombinant monoclonal antibodies (MAbs) are increasingly being used as therapeutic agents, especially in combination with anti-cancer drugs. Second, conjugation of antibody therapy with toxins or radioisotopes offers more therapeutic approaches. Third, development of cytotoxic T-lymphocyte (CTL) or natural killer (NK)-cell populations with anti-TAA antibody activity may be important for the success of cancer immunotherapy because the downregulated HLA class I molecules and the non-ubiquitous expression of NK receptor ligands in tumor tissues constitute the major tumor escape mechanism facing tumor-specific CTL- and/or NK-cell-mediated responses. Finally, in cancer gene therapy, the strategies to target viral vectors carrying therapeutic genes to tumor tissues by modifying the tropisms with MAbs or their genes against TAAs are also very promising.

There are many tumor-associated antigens (TAAs) against which monoclonal antibodies (MAbs) have been widely tested as treatment for cancer (1). Several specific immunotherapies using anti-TAA antibodies seem to be very promising, because the actual expression of antibody-recognized TAAs on target tumor cells is easily confirmed

by immunostaining and/or flow cytometry with the corresponding antibodies. Clinical benefit is more likely if the TAAs are expressed only on cancer cells and not on vital tissue. The major antibody-recognized TAAs used as the targets of cancer therapy are CD20 (2) or CD22 for B-cell non-Hodgkin's lymphoma (3), CD33 for acute myeloid leukemia (4), CD52 for chronic lymphocytic leukemia (5), human epidermal growth factor receptor 2 (HER2, Her-2/neu or *c-erbB-2*) for breast cancer (6), epidermal growth factor receptor (EGFR) for colorectal or lung cancer (7), vascular endothelial growth factor (VEGF) for colorectal cancer (8), carcinoembryonic antigen (CEA) for various cancers including gastrointestinal cancer (9, 10), CA72-4 (TAG-72) for gastrointestinal cancer (11), epithelial cell adhesion molecule (EpCAM) (also known as 17-1A) for colorectal cancer (12), high-molecular weight melanoma-associated antigen (HMWMAA) for malignant melanoma (13), etc. This article provides a brief overview of the possible applications of anti-TAA antibodies in cancer therapy (Table I).

Therapy with Native Antibody

Mechanisms of unconjugated, native antibody-based therapy range from the engagement of immune effector mechanisms such as complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) to the anti-proliferation effect and apoptosis induction by the blockade of receptor/ligand interactions (14) (Table I).

Early attempts at immunotherapy using polyclonal antibodies have been limited due to the difficulty of achieving high titre and specificity. The advent of MAbs promises to overcome these difficulties (15). Furthermore, in order to overcome immunogenic problems associated with the administration of mouse MAbs (with the suffix "-

Correspondence to: Dr. Masahide Kuroki, Department of Biochemistry, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka, 814-0180, Japan. Tel: +81-92-801-1011 Ext. 3240, Fax: +81-92-801-3600, e-mail: kurokima@fukuoka-u.ac.jp

Key Words: Cancer, gene therapy, immunotherapy, monoclonal antibodies, tumor-associated antigen, review.

Table I. Possible applications of anti-TAA antibodies or their genes in cancer therapy.

- I. Therapy with native antibody (Ab alone or in combination with chemotherapy)
 1. Cell killing mechanism: CDC, ADCC or blockade of receptor/ligand interactions
 2. Antibodies
 - 1) Mouse monoclonal antibody (-momab)
 - 2) Mouse/human chimeric antibody (-ximab): rituximab (Rituxan™), cetuximab Erbitux™
 - 3) Humanized antibody (-zumab): Trastuzumab (Herceptin™), alemtuzumab (Campath™), bevacizumab (Avastin™)
 - 4) Human antibody (-umab)
- II. Therapy with conjugated antibody (Anticancer agents conjugated to antibody)
 1. Cell killing mechanism: anticancer effects of drugs
 2. Conjugated antibodies
 - 1) Toxin-conjugated antibody: gemtuzumab (Mylotarg™)
 - 2) Chemotherapeutic-conjugated antibody
 - 3) Radioisotope-conjugated antibody: ibritumomab (Zevalin™), tositumomab (Bexxar™)
 - 4) Prodrug-conjugated antibody
 - 5) Photosensitizer-conjugated antibody
- III. Fusion therapy with cellular immunity (Antibody-directed CTLs or NK-cells)
 1. Cell killing mechanism: cellular immunity
 2. Fusion strategies
 - 1) Bispecific antibody
 - 2) Antibody-cytokine fusion protein
 - 3) Antibody-TCR chimeric immune receptor
 - 4) Antibody-HLA/peptide complex
- IV. Antibody-targeted gene therapy (Antibody-directed viral vectors)
 1. Cell killing mechanism: anticancer effects of therapeutic gene products
 2. Vectors
 - 1) Antibody-directed retroviral vector
 - 2) Antibody-directed adenoviral vectors

momab" in their international nonproprietary names), mouse-human chimeric antibodies ("-ximab") are produced by genetically fusing the mouse variable domain to human constant domains. The anti-CD20 MAb rituximab (Rituxan™) is an example of a chimeric antibody widely used in the treatment of non-Hodgkin's lymphoma (Table I) (16). Although chimeric antibodies possess reduced immunogenicity, they sometimes elicit a significant human anti-chimeric antibody response. To address this problem, the complementary determining regions, which are responsible for antigen binding within the variable regions, have been transferred to human frameworks, thereby creating humanized antibodies ("-zumab") (17). Trastuzumab (Herceptin™) and alemtuzumab (Campath™) are two examples of humanized MABs commonly used in

the treatment of patients with metastatic breast cancers overexpressing HER2 (6) and with chronic lymphocytic leukemia (5), respectively (Table I). Thus, the use of MAB therapy for the treatment of human cancer has recently advanced from experimental level to standard care for some malignancies (18). Moreover, it has become easy to generate fully human MABs ("-umab") to any human TAAs using the KM mouse™ and usual hybridoma techniques. The KM mouse™ has been produced by the combination of mouse Ig-knockout and human Ig-transgenic technologies (19). More recently, 46 fully human mAbs to CEA using the KM mouse™ have been generated (20). Among them, 22 clones reacted with CEA but not with other CEA gene family members. Considering their complete lack of immunogenicity to humans, these CEA-specific human MABs may be useful for immunotherapeutic approaches.

Antibody therapy has also been studied in combination with anticancer chemotherapeutic drugs (18) (Table I). In a randomized phase III trial comparing chemotherapy alone (anthracycline and cyclophosphamide) or chemotherapy plus trastuzumab as the initial treatment in women with HER2-positive metastatic breast cancer, trastuzumab was found to increase the clinical benefit of chemotherapy (21). While the above study assessed trastuzumab in combination with adriamycin and cytoxan or paclitaxel, preclinical studies have indicated that the antibody may also be synergistic with other chemotherapeutic agents, such as carboplatin, cyclophosphamide, docetaxel, and vinorelbine (22). VEGF, which resides in the vasculature that feeds tumor cells and seems to be correlated with the risk of metastases, has become the first anti-angiogenic target to be exploited. Bevacizumab (Avastin™) is a humanized MAB that blocks binding of the VEGF to its receptor on vascular endothelium (23) (Table I). In a randomized trial involving patients with previously untreated metastatic colorectal cancer, the chemotherapy plus antibody group received irinotecan, fluorouracil, and leucovorin plus bevacizumab and the control group received the same three chemotherapeutics plus placebo (8). The median survival was 20.3 months in the experimental group *versus* 15.6 months in the placebo group. The overall response rates were also higher in the antibody treated group. This study has led to the FDA approval of bevacizumab for the treatment of patients with metastatic colorectal cancer.

Therapy with Drug-conjugated Antibody

MABs that are not capable of directly eliciting antitumor effects, either by directing immune system cells or altering signal transduction, can still be effective against tumors as selective carriers for delivering cytotoxic agents such as toxins, chemotherapeutics, radioisotopes, prodrugs, and photosensitizers (Table I) (1, 24).

Immunotoxins are antibodies or antibody fragments that are conjugated to a toxin and are designed to deliver the toxin to the cell surface (25). The therapeutic goal is to deliver the toxin to the surface of the cancer cells *via* antibody binding to a target, and after internalization of the toxin to cause cell death. Toxins can be derived from plants or bacteria. They can be small molecules, such as calicheamicins (25), or toxins, such as the pseudomonas or diphtheria toxin. Mylotarg™ (gemtuzumab), an anti-CD33 MAb conjugated to calicheamicin, is an immunotoxin approved for use in patients with recurrent or refractory acute myeloid leukemia (Table I) (4).

So far, two radioimmunoconjugates have been approved for use in clinical practice by the FDA, Zevalin™ (ibritumomab) and Bexxar™ (tositumomab) (23) (Table I). Both of these agents are approved for use in patients with recurrent or refractory non-Hodgkin's lymphoma. Zevalin is an Yttrium-90 labeled anti-CD20 MAb that has demonstrated effectiveness for the treatment of non-Hodgkin's lymphoma (26). Indeed, treatment with this agent has yielded very impressive rates of response and prolonged durations of complete remission in patients with favorable, low-grade indolent lymphoma when used as initial therapy (27). Although the use of radioimmunotherapy has proved to be useful in the treatment of patients with lymphomas, patients with solid tumors have not benefited from this approach. This could be related to either lack of tumor penetration or incomplete tumor targeting with currently available antibody constructs. The radiolabeled chimeric anti-CD20 MAb (rituximab) or humanized anti-CD22 MAb (epratuzumab) have also shown promise against B-cell lymphomas resistant to conventional therapy (28, 29).

In photodynamic therapy for cancer, MAbs against TAAs conjugated with photosensitizers are utilized to increase tumor specificity. This approach is called photoimmunotherapy (30). Recently, a conjugate between a novel mouse MAb (F11-39) against-CEA and a new photosensitizer (ATX-70) has been synthesized (31). When applied to a mouse xenograft model, this conjugate exhibited marked growth inhibition of CEA-expressing human tumor cells in comparison to irradiation alone or irradiation after administration of ATX-70.

Fusion Therapy with Cellular Immunity

Cellular immunity, in which cytotoxic T-lymphocytes (CTLs) and natural killer (NK)-cells are the main effector cells, plays an important role in the anti-tumor defense mechanism. T-cell immunotherapy is based on the assumption that TAA peptides are correctly presented by HLA class I molecules on target tumor cells, and NK-cell immunotherapy is based on the hypothesis that cell surface TAAs or ligands for NK receptors are widely expressed in tumor cells. However, it is well known that human tumor cells often lose HLA class I

molecules, and target cell ligands for NK receptors are not always expressed in human tumor cells. The altered HLA class I expression and nonubiquitous distribution of NK receptor ligands constitute the major tumor escape mechanism facing tumor-specific CTL and/or NK-cell mediated responses. Therefore, endowing CTLs or NK-cells with antigen-binding specificity of anti-TAA antibody is a promising approach for re-targeting the activities of these effector cells to tumor cells in an HLA-independent manner. The following four strategies are currently being tried as fusion therapy between anti-TAA antibody and CTLs/NK-cells (Table I) (32).

Bispecific antibody technology allows the generation of a single antibody directed against both a TAA and a given surface marker on effector cells, such as CD3 on T-cells or CD16 on NK-cells (33). In theory, these antibodies should help to localize the lymphokine-activated killer (LAK)-cells on the tumor *via* their anti-TAA activity. LAK-cells consist mainly of NK (NK-LAK)- and/or CTL (T-LAK)-cells (34). Recently, Reusch *et al.* have generated a bispecific antibody consisting of anti-CD3 (OKT3) and anti-EGFR (cetuximab, Erbitux™) (Table I) (7). The bispecific antibody was able to redirect T-LAK activity to human EGFR-positive cancers *in vitro* and in an animal model. In addition, Grabert *et al.* produced an anti-CD3 (OKT3) x anti-HER2 (trastuzumab) bispecific antibody and showed that human T-LAK-cells armed with the bispecific antibody were cytotoxic and secreted cytokines with repeated stimulation (35). Taken together, these bispecific antibodies may serve as a potentially useful immunotherapeutic reagent for human TAA-expressing cancers.

The fusion of anti-TAA mAbs and cytokines is an efficient technique for targeting cytokines to tumor cells, and hence focusing the killing activity of LAK-cells *via* cytokine receptors to the target cells (9, 36). In a previous study, Xu *et al.* generated an anti-CEA antibody-IL-2 fusion protein for selective tumor targeting of cytokines (9). The variable domains of a high affinity anti-CEA antibody were used to form a single-chain variable fragmented (scFv) antibody joined to the crystallizable fragment, Fc (scFvFc). The fusion protein, designated scFvFc. IL-2, consisted of mouse IL-2 fused to the C-terminal end of the scFvFc. The growth of CEA-expressing syngeneic tumor cells in the CEA-transgenic mouse model was inhibited after treatment with scFvFc.IL-2. We have also genetically fused human IL-2 to the F11-39 scFv antibody to CEA (37). The resulting fusion protein effectively targeted IL-2 onto the surface of CEA-expressing tumor cells and consequently introduced the specific cytotoxicity of human NK-LAK-cells to the tumor cells.

Chimeric immune receptor (CIR) technology also has the potential to re-target CTLs or NK-cells to tumor cells (38). Recombinant CIRs encompass anti-TAA antibodies genetically fused to the signaling domains of either T-cell receptor (TCR) or Fc receptor (FcR). After transfection,

CTLs or NK-cells expressing anti-TAA scFv/TCR- ζ (CD3 ζ) or anti-TAA scFv/FcR γ receptors amplify the cytopathic effects mediated by TCR or FcR and allow targeting to tumor cells in an HLA-independent manner (39). Furthermore, given that T-cells require both primary and co-stimulatory signals for optimal activation and that many tumors do not express critical co-stimulatory ligands, modified CIRs have been engineered to co-deliver CD28 co-stimulation (40). Thanks to this CIR technology, large numbers of T-LAK- or NK-LAK-cells with redirected anti-CEA specificity have thus been generated. We also have recently constructed a CIR gene that encoded the F11-39 scFv antibody to CEA, the human CD8 α hinge region, the CD28 transmembrane and cytoplasmic domains, and the human CD3 ζ -chain (41). Haynes *et al.* have compared the antitumor potency of primary T-lymphocytes expressing CEA-reactive CIRs that incorporate either TCR- ζ (CD3 ζ) or CD28/CD3 ζ signaling (40). Although both receptor-transduced T-cell effector populations demonstrated cytotoxicity of CEA-expressing tumors *in vitro*, T-cells expressing the scFv/CD28/CD3 ζ chimera had a far greater capacity to control the growth of CEA-expressing xenogeneic or syngeneic colon carcinomas *in vivo*. Overall, this study illustrated the ability of a chimeric scFv receptor capable of harnessing the signaling machinery of both TCR- ζ and CD28 to augment T-cell immunity against tumors that have lost expression of both MHC/peptide and co-stimulatory ligands *in vivo*.

Recently, several anti-TAA antibody-HLA-restricted antigen peptide complexes have been constructed for antibody-guided re-targeting of relevant T-LAK-cells towards tumors (42-44). First, Robert *et al.* have successfully targeted flu-sensitized T-LAK-cells to tumor cells by pulsing with anti-TAA Fab'-HLA/flu peptide complexes (42, 43). Single Fab' fragments from an anti-CEA mAb were coupled to either tetrameric or monomeric HLA-A2 complexes containing the immunodominant influenza-matrix peptide 58-66. When targeted to CEA-expressing tumor cells, the conjugates induced CTL activation and efficient tumor cell lysis *in vitro*, as a result of HLA/flu peptide surface oligomerization, independent of the HLA class I molecule expression on target tumor cells. Furthermore, *in vivo* targeting of the anti-CEA Fab'-HLA/flu peptide complexes induced specific growth inhibition and regression of established syngeneic tumor grafts (45). More recently, Lev *et al.* have demonstrated that targeting an anti-TAA scFv-HLA/peptide complex to tumor cells could function *in vitro* and most significantly *in vivo* in a human solid xenograft tumor model (46). These results suggest that injection of Fab'-HLA/flu peptide conjugates could represent a new form of immunotherapy, bridging antibody and influenza-sensitized CTL attack on cancer cells.

Antibody-targeted Gene Therapy

Cancer gene therapy is one of the main applications of gene therapy. In the past decade, both viral and non-viral vectors have been developed and evaluated for delivering therapeutic genes that can eliminate tumor cells. In the last few years, numerous modifications to the delivery systems have been made to optimize the transfection efficacy. Among them, the following two strategies to target viral vectors to tumor tissues by modifying the tropisms with antibodies or their genes against TAAs are very promising from a practical point of view (Table I) (47).

Retrovectors remain an attractive option for clinical gene delivery because integration of the vector genome allows stable gene expression in the infected cell and its progeny (48). The retrovectors used for most clinical trials of gene therapy originate from a murine leukemia virus (MLV). Because viral coding regions are deleted from the vector, viral proteins are not expressed in the infected cells, avoiding stimulation of an inappropriate immune response. Also, the host range of retrovectors is usually determined by the surface domain of the envelope glycoprotein, which covers the viral capsid and binds to a cell surface receptor. As retrovectors transduce only dividing cells, they have been used to deliver therapeutic genes to tumors *in vivo*, with surrounding normal tissue being largely refractory to transduction (49). Recently, however, a patient with X-linked severe combined immunodeficiency, who received gene therapy using retrovirally transduced bone marrow cells, developed T-cell leukemia caused by retrovector integration leading to insertional mutagenesis (50). This highlights the need to target retroviral gene delivery specifically to tumors, if vectors or packaging cells are to be injected *in vivo* for cancer gene therapy. Recent advances in the field of genetic engineering have led to development of a concept for target cell specificity by modifying the tropism of the normal envelope, retroviral receptor-binding domain with an scFv antibody or a ligand that recognizes a TAA (51) or a specific cell surface receptor (52). The major antibody-recognized TAAs currently used as the targets are CEA (10, 49) and HMWMAA (13). In a recent study, a novel bifunctional MLV-based recombinant retrovector that displays a chimeric envelope protein containing an scFv antibody to CEA and carries a suicide, inducible nitric oxide synthase (iNOS) gene into the genome has been developed (10). The MLV-based retrovector used here is ecotropic, and originally infects only murine cells. The iNOS gene product yields nitric oxide, which directly induces autotoxicity and cytotoxicity of by-stander cells. An anti-CEA scFv antibody derived from the mouse hybridoma F11-39 was genetically inserted into the ecotropic envelope protein. The resultant bifunctional retrovector, GPESCv-env/iNOS, showed a specific delivery of the iNOS gene to

human CEA-expressing tumor cells (MKN-45 gastric carcinoma cells) and directly and efficiently killed the infected CEA-expressing tumor cells by the induction of apoptosis without any additional drugs. The targeted vector was able to produce tumor suppression in a mouse xenograft model with 70% reduction in tumor weight (53). This result suggests that a tumor-specific therapeutic effect could be achieved by using the scFv-chimeric retroviral envelope protein model to deliver suicide genes *in vivo* and this approach could also be applied to other TAAs expressing on cancer cells.

Adenovectors are also promising reagents for clinical gene delivery because of their superior *in vivo* gene transfer efficiency on a wide spectrum of cell types and their low risk of mutagenesis. Adenovectors, like adenoviruses, do not have an envelope and their major capsid components are hexon, penton (or penton base), and knobbed fiber (fiber and fiber knob). Adenoviral infection is mediated by binding of the knob region, located at the carboxy terminus of the fiber, to its corresponding receptor, which is the coxsackie-adenovirus receptor (CAR) (54). Binding is followed by interaction between cellular integrins and an arginine-glycine-aspartic acid (RGD) motif located at the penton base. Infection is not dependent on cell cycle phase; therefore, both cycling and non-dividing cells are infected, and adenoviral DNA is not integrated into the host genome. Although the limited duration of gene expression may render adenovectors less desirable for the gene therapy of hereditary diseases where long-term expression is needed, it is adequate for cancer gene therapy approaches where the primary purpose is to kill the target cells (54). However, adenovectors should also possess critical properties required for the development of efficient and targeted gene transfer vectors for the successful clinical translation of cancer gene therapy (55). These include a highly evolved gene transfer mechanism, the stability of virus particles and the ease of virus production at high titers. The necessity of such improvement is predicated by the observation that CAR is widely expressed on normal tissues resulting in nonspecific susceptibility to adenoviral infection. In addition, reduced or absent expression of CAR has been reported for various tumor types, indicating resistance to adenoviral infection by tumor cells *in situ*. These considerations of adenoviral biology are paralleled by the observation of limited efficacy and vector-related toxicity in preclinical and clinical adenoviral gene therapy studies. Therefore, the development of tropism-modified, tumor-targeted adenovectors is a key endeavor in current gene therapy approach. To this end, the native tropism of adenoviruses needs to be ablated and a new, tumor-specific tropism needs to be engineered into viral particles (55). Trials have been performed in several ways among

which are: a) fusion protein of soluble CAR (sCAR) and targeting-receptor ligand (56); b) fusion protein of anti-fiber knob antibody and targeting-receptor ligand (57); c) bispecific antibody to fiber knob and TAA (or cell receptor) (12); d) fusion protein of sCAR and scFv antibody to TAA (58); and e) immunoglobulin-binding domain inserted fiber-knob protein (59). The antibody-recognized TAAs (or cell receptors) used as the targets include HMWMAA (55), EpCAM (12), EGFR (59) and HER2 (58). Recently, a novel modified adenovector that displays a synthetic IgG-binding domain in the capsid and carries a reporter *lacZ* gene has been developed (60). A synthetic 33-amino-acid IgG-binding domain (Z33), derived from staphylococcal protein A, was inserted into the adenovirus fiber protein. The fiber retained the ability to assemble into trimers, bound IgG with high affinity, and was incorporated into vector particles. The transduction efficiency of the Z33-modified adenovector in human CEA-expressing tumor cells (MKN-45 cells) was strongly and dose-dependently enhanced by combination with a CEA-specific monoclonal antibody. The antibody-mediated increase in cellular transduction was abolished in the presence of competing protein A. Thus, the IgG-binding adenovector holds promise for directed gene transfer to a wide variety of cell types by simply changing the target-specific antibody.

References

- 1 Kuroki Ma, Ueno A, Matsumoto H, Abe H, Li T, Imakiire T, Yamauchi Y, Uno K, Shirota K, Shibaguchi H *et al*: Significance of tumor-associated antigens in the diagnosis and therapy of cancer: an overview. *Anticancer Res* 22: 4255-4264, 2002.
- 2 McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F *et al*: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 16: 2825-2833, 1998.
- 3 Leonard JP, Coleman M, Ketas JC, Chadburn A, Ely S, Furman RR, Wegener WA, Hansen HJ, Ziccardi H, Eschenberg M *et al*: Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J Clin Oncol* 21: 3051-3059, 2003.
- 4 Sievers EL, Appelbaum FR, Spielberger RT, Forman SJ, Flowers D, Smith FO, Shannon-Dorcy K, Berger MS and Bernstein ID: Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate. *Blood* 93: 3678-3684, 1999.
- 5 Mone AP, Cheney C, Banks AL, Tridandapani S, Mehter N, Guster S, Lin T, Eisenbeis CF, Young DC and Byrd JC: Alemtuzumab induces caspase-independent cell death in human chronic lymphocytic leukemia cells through a lipid raft-dependent mechanism. *Leukemia* 20: 272-279, 2006.

- 6 Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G *et al*: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 17: 2639-2648, 1999.
- 7 Reusch U, Sundaram M, Davol PA, Olson SD, Davis JB, Demel K, Nissim J, Rathore R, Liu PY and Lum LG: Anti-CD3 x anti-epidermal growth factor receptor (EGFR) bispecific antibody redirects T-cell cytolytic activity to EGFR-positive cancers *in vitro* and in an animal model. *Clin Cancer Res* 12: 183-190, 2006.
- 8 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E *et al*: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *New Engl J Med* 350: 2335-2342, 2004.
- 9 Xu X, Clarke P, Szalai G, Shively JE, Williams LE, Shyr Y, Shi E and Primus FJ: Targeting and therapy of carcinoembryonic antigen-expressing tumors in transgenic mice with an antibody-interleukin 2 fusion protein. *Cancer Res* 60: 4475-4484, 2000.
- 10 Khare PD, Liao S, Kuroki Mo, Hirose Y, Arakawa F, Nakamura K, Tomita Y and Kuroki Ma: Specifically targeted killing of carcinoembryonic antigen (CEA)-expressing cells by a retrovector displaying single chain variable fragmented (scFv) antibody to CEA and carrying inducible nitric oxide synthase (iNOS) gene. *Cancer Res* 61: 370-375, 2001.
- 11 Milenic D, Garmentani K, Dadachova E, Chappell L, Albert P, Hill D, Schlom J and Brechbiel M: Radioimmunotherapy of human colon carcinoma xenografts using a ²¹³Bi-labeled domain-deleted humanized monoclonal antibody. *Cancer Biother Radiopharm* 19: 135-147, 2004.
- 12 Haisma, HJ, Pinedo HM, van Rijswijk A, van der Muelen Muileman I, Sosnowski BA, Ying W, van Heusechem VW, Tillman BW, Gerritsen WR and Curiel DT: Tumor-specific gene transfer *via* an adenoviral vector targeted to the pancreatic carcinoma antigen EpCAM. *Gene Ther* 6: 1469-1474, 1999.
- 13 Martin F, Chowdhury S, Neil SJ, Chester KA, Cosset FL and Collins MK: Targeted retroviral infection of tumor cells by receptor cooperation. *J Virol* 77: 2753-2756, 2003.
- 14 Prang N, Preithner S, Brischwein K, Goster P, Woppel A, Muller J, Steiger C, Peters M, Baeuerle PA and Da Silva AJ: Cellular and complement-dependent cytotoxicity of Ep-CAM-specific monoclonal antibody MT201 against breast cancer cell lines. *Br J Cancer* 92: 342-349, 2005.
- 15 Stocchi L and Nelson H: Diagnostic and therapeutic applications of monoclonal antibodies in colorectal cancer. *Dis Colon Rectum* 41: 232-250, 1998.
- 16 Czuczman MS, Grillo-Lopez AJ, White CA, Saleh M, Gordon L, LoBuglio AF, Jonas C, Klippenstein D, Dallaire B and Varns C: Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol* 17: 268-276 1999.
- 17 Wright A, Shin SU and Morrison SL: Genetically engineered antibodies: progress and prospects. *Crit Rev Immunol* 12: 125-168, 1992.
- 18 Truica CI, Rouch D and Arteaga CL: Antibody therapy for solid tumors. In: *Immunotherapy of cancer*. Disis ML (ed.). Totowa (NJ, USA), Humana Press, pp. 433-444, 2006.
- 19 Ishida I, Tomizuka K, Yoshida H, Tahara T, Takahashi N, Ohguma A, Tanaka S, Umehashi M, Maeda H, Nozaki C, Halk E and Lonberg N: Production of human monoclonal and polyclonal antibodies in TransChromo animals. *Cloning Stem Cells* 4: 91-102, 2002.
- 20 Imakiire T, Kuroki Mo, Shibaguchi H, Abe H, Yamauchi Y, Ueno A, Hirose Y, Yamada H, Yamashita Y, Shirakusa T, Ishida I and Kuroki Ma: Generation, immunologic characterization and antitumor effects of human monoclonal antibodies for carcinoembryonic antigen. *Int J Cancer* 108: 564-570, 2004.
- 21 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J and Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New Engl J Med* 344: 783-792, 2001.
- 22 Pegram MD, Konecny GE, O'Callaghan C, Beryt M, Pietras R and Slamon DJ: Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. *J Natl Cancer Inst* 96: 739-749 2004.
- 23 Borghaei H, Robinson MK and Weiner LM: Monoclonal antibody therapy of cancer. In: *Immunotherapy of cancer*. Disis ML (ed.). Totowa (NJ, USA), Humana Press, pp. 487-502, 2006.
- 24 Allen TM: Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* 2: 750-763, 2002.
- 25 Presta LG: Engineering antibodies for therapy. *Curr Pharm Biotechnol* 3: 237-256, 2002.
- 26 Witzig TE, White CA, Wiseman GA, Gordon LI, Emmanouilides C, Raubitschek A, Janakiraman N, Gutheil J, Schilder RJ, Spies S *et al*: Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 17: 3793-3803, 1999.
- 27 Kaminski MS, Tuck M, Estes J, Kolstad A, Ross CW, Zasadny K, Regan D, Kison P, Fisher S, Kroll S and Wahl RL: 131I-tositumomab therapy as initial treatment for follicular lymphoma. *New Engl J Med* 352: 441-449, 2005.
- 28 McLaughlin P: Biotherapy for lymphoma. *Curr Oncol Rep* 2: 157-162, 2000.
- 29 White CA, Weaver RL and Grillo-Lopez AJ: Antibody-targeted immunotherapy for treatment of malignancy. *Annu Rev Med* 52: 125-145, 2001.
- 30 Duska LR, Hamblin MR, Miller JL and Hasan T: Combination photoimmunotherapy and cisplatin: effects on human ovarian cancer *ex vivo*. *J Natl Cancer Inst* 91: 1557-1563, 1999.
- 31 Abe H, Kuroki M, Tachibana K, Li T, Awasthi A, Ueno A, Matsumoto H, Imakiire T, Yamauchi Y, Yamada H, Ariyoshi A and Kuroki Ma: Targeted sonodynamic therapy of cancer using a photosensitizer conjugated with antibody against carcinoembryonic antigen. *Anticancer Res* 22: 1575-1580; 2002.
- 32 Kuroki Ma, Hachimine K, Huang J, Shibaguchi H, Kinugasa T, Maekawa S and Kuroki Mo: Re-targeting of cytotoxic T lymphocytes and/or natural killer cells to CEA-expressing tumor cells with anti-CEA antibody activity. *Anticancer Res* 25: 3725-3732, 2005.
- 33 Bolhuis RL, Sturm E and Braakman E: T cell targeting in cancer therapy. *Cancer Immunol Immunother* 34: 1-8, 1991.
- 34 Yamamoto T, Yoneda K, Osaki T, Yoshimura N and Akagi N: Longer local retention of adoptively transferred T-LAK cells correlates with lesser adhesion molecule expression than NK-LAK cells. *Clin Exp Immunol* 100: 13-20, 1995.

- 35 Grabert RC, Cousens LP, Smith JA, Olson S, Gall J, Young WB, Davol PA and Lum LG: Human T cells armed with Her2/neu bispecific antibodies divide, are cytotoxic, and secrete cytokines with repeated stimulation. *Clin Cancer Res* 12: 569-576, 2006.
- 36 Penichet ML and Morrison SL: Antibody-cytokine fusion proteins for the therapy of cancer. *J Immunol Methods* 248: 91-101, 2001.
- 37 Liao S, Khare PD, Arakawa F, Kuroki M, Hirose Y, Fujimura S, Tomita Y and Kuroki Ma: Targeting of LAK activity to CEA-expressing tumor cells with an anti-CEA scFv/IL-2 fusion protein. *Anticancer Res* 21: 1673-1680, 2001.
- 38 Kuwana Y, Asakura Y, Utsunomiya N, Nakanishi M, Arata Y, Itoh S, Nagase F and Kurosawa Y: Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. *Biochem Biophys Res Commun* 149: 960-968, 1987.
- 39 Patel SD, Moskalenko M, Smith D, Maske B, Finer MH and McArthur JG: Impact of chimeric immune receptor extracellular protein domains on T cell function. *Gene Ther* 6: 412-419, 1999.
- 40 Haynes NM, Trapani JA, Teng MW, Jackson JT, Cerruti L, Jane SM, Kershaw MH, Smyth MJ and Darcy PK: Rejection of syngeneic colon carcinoma by CTLs expressing single-chain antibody receptors codelivering CD28 costimulation. *J Immunol* 169: 5780-5786, 2002.
- 41 Arakawa F, Shibaguchi H, Xu Z and Kuroki Ma: Targeting of T cells to CEA-expressing tumor cells by chimeric immune receptors with a highly specific single-chain anti-CEA activity. *Anticancer Res* 22: 4285-4290, 2002.
- 42 Robert B, Guillaume P, Luescher I, Romero P and Mach J P: Antibody-conjugated MHC class I tetramers can target tumor cells for specific lysis by T lymphocytes. *Eur J Immunol* 30: 3165-3170, 2000.
- 43 Robert B, Guillaume P, Luescher I, Doucey MA, Cerottini JC, Romero P and Mach J-P: Redirecting anti-viral CTL against cancer cells by surface targeting of monomeric MHC class I-viral peptide conjugated to antibody fragments. *Cancer Immunity* 1: 2, 2001.
- 44 Lev A, Novak H, Segal D and Reiter Y: Recruitment of CTL activity by tumor-specific antibody-mediated targeting of single-chain class I MHC-Peptide Complexes. *J Immunol* 169: 2988-2996, 2002.
- 45 Donda A, Cesson V, Mach J-P, Corradin G, Primus FF and Robert B: *In vivo* targeting of an anti-tumor antibody coupled to antigenic MHC class I complexes induces specific growth inhibition and regression of established syngeneic tumor grafts. *Cancer Immunity* 3: 11, 2003.
- 46 Lev A, Noy R, Oved K, Novak H, Segal D, Walden P, Zehn D and Reiter Y: Tumor-specific Ab-mediated targeting of MHC-peptide complexes induces regression of human tumor xenografts *in vivo*. *Proc Natl Acad Sci USA* 101: 9051-9056, 2004.
- 47 Kuroki Ma, Shibaguchi H, Kuroki Mo, Kinugasa T, Hachimine K, Enatsu S, Maekawa S, Huang J and Zhao J: Targeting of cancer gene therapy with antibodies or their genes against tumor-associated antigens. *Gene Ther Mol Biol* 9: 107-112, 2005.
- 48 Hunt KK and Vorbuerger SA: Hurdles and hopes for cancer treatment. *Science* 297: 415-416, 2002.
- 49 Chowdhury S, Chester KA, Bridgewater J, Collins MK and Martin F: Efficient retroviral vector targeting of carcinoembryonic antigen-positive tumors. *Mol Ther* 9: 85-92, 2004.
- 50 Kohn DB, Sadelain M and Glorioso JC: Occurrence of leukaemia following gene therapy of X-linked SCID. *Nat Rev Cancer* 3: 477-488, 2003.
- 51 Somia NV, Zoppe M and Verma IM: Generation of targeted retroviral vectors by using single-chain variable fragment: an approach to *in vivo* gene delivery. *Proc Natl Acad Sci USA* 92: 7570-7574, 1995.
- 52 Kasahara N, Dozy AM and Kan YW: Tissue-specific targeting of retroviral vectors through ligand-receptor interactions. *Science* 266: 1373-1376, 1994.
- 53 Khare PD, Liao S, Hirose Y, Kuroki Mo, Fujimura S, Yamauchi Y, Miyajima-Uchida H and Kuroki Ma: Tumor growth suppression by a retroviral vector displaying scFv Antibody to CEA and Carrying the iNOS Gene. *Anticancer Res* 22: 2443-2446, 2002.
- 54 Bauerschmitz GJ, Barker SD and Hemminki A: Adenoviral gene therapy for cancer: from vectors to targeted and replicativecompetent agents (Review). *Int J Oncol* 21: 1161-1174, 2002.
- 55 Nettelbeck DM, Rivera AA, Kupsch J, Dieckmann D, Douglas JT, Kontermann RE, Alemany R and Curiel DT: Retargeting of adenoviral infection to melanoma: combining genetic ablation of native tropism with a recombinant bispecific single-chain diabody (scDb) adapter that binds to fiber knob and HMWMAA. *Int J Cancer* 108: 136-145, 2004.
- 56 Dmitriev I, Kashentseva E, Rogers BE, Krasnykh V and Curiel DT: Ectodomain of coxsackievirus and adenovirus receptor genetically fused to epidermal growth factor mediates adenovirus targeting to epidermal growth factor receptor-positive cells. *J Virol* 74: 6875-6884, 2000.
- 57 Watkins SJ, Mesyanzhinov VV, Kurochkina LP and Hawkins RE: The 'adenobody' approach to viral targeting: specific and enhanced adenoviral gene delivery. *Gene Ther* 4: 1004-1012, 1997.
- 58 Kashentseva EA, Seki T, Curiel DT and Dmitriev IP: Adenovirus targeting to c-erbB-2 oncoprotein by single-chain antibody fused to trimeric form of adenovirus receptor ectodomain. *Cancer Res* 62: 609-616, 2002.
- 59 Volpers C, Thirion C, Biermann V, Hussmann S, Kewes H, Dunant P, Von Der Mark H, Herrmann A, Kochanek and Lochmuller H: Antibody-mediated targeting of an adenovirus vector modified to contain a synthetic immunoglobulin G-binding domain in the capsid. *J Virol* 77: 2093-2104, 2003.
- 60 Tanaka T, Huang J, Hirai S, Kuroki Mo, Kuroki Ma, Watanabe N, Kato K and Hamada H: CEA-targeted selective gene therapy for gastric cancer through FZ33 fiber modified adenovirus vector. *Clin Cancer Res* 12: 3803-3813, 2006.

Received May 23, 2006

Revised July 13, 2006

Accepted July 18, 2006